Appendix E

TRE Case Study: Michigan City Sanitary District, Indiana

Abstract

TRE Goal: LC50 ≥ 100%, NOEC ≥ 62%
Test Organisms: *C. dubia* and *P. promelas*
TRE Elements: TIE
Toxicant Identified: Metals
Toxicity Controls: Pretreatment requirements

Summary

Acute and chronic TIE studies indicated that metals were the primary cause of effluent toxicity. An industrial user was identified as a major source of metals loadings to the POTW. The POTW staff required the industrial user to discontinue a cadmium plating operation and, as a result, the POTW effluent has achieved compliance with the acute and chronic toxicity limits (MCSD, 1993).

Key Elements

1. Less expensive acute TIE procedures can be used in lieu of chronic TIE procedures to help characterize the causes of chronic effluent toxicity. However, chronic TIE testing is needed to confirm the acute TIE results.
2. C18 SPE can remove toxicity caused by compounds other than non-polar organic compounds. In this study, C18 SPE treatment removed toxicity caused by metals. These results demonstrate the importance of needing to recover toxicity from the C18 SPE column before concluding that non-polar organic compounds are causing effluent toxicity.
3. TIE Phase I data may provide sufficient information to proceed to the selection of pretreatment controls for toxicity reduction. Although specific toxic metals were not identified in this study, evidence of metals toxicity was successfully used to set pretreatment requirements.

Introduction

Permit Requirements

The NPDES permit for the Michigan City Wastewater Treatment Plant (MCWTP) requires acute and chronic toxicity monitoring using *C. dubia* and *P. promelas*. The permit specifies that the effluent must not demonstrate chronic effluent toxicity at effluent concentrations of 62% or less (≤ 1.6 TUc) and that the effluent must not be acutely toxic (e.g., LC50 ≥ 100%, ≤ 1.0 TUa). Based on evidence of unacceptable acute and chronic toxicity, Michigan City was required to perform a TRE. The Michigan City Sanitary District submitted a TRE plan and initiated TIE testing. The objective of the TIE was to characterize, identify, and confirm the causes of acute and chronic effluent toxicity so that an appropriate toxicity reduction strategy could be developed and implemented.

Description of Treatment Plant

The MCWTP comprises an activated sludge process with single-stage nitrification and advanced waste treatment of the secondary effluent. The facility is designed for an average wastewater flow of 12-million gallons per day (mgd) and 96.7% removal of BOD_5 and 96% removal of suspended solids. Monthly average effluent limits for ammonia are 2 mg/L in summer and 6 mg/L in the winter. Influent phosphorus is reduced with an iron salt added at the aeration tanks. Additional phosphorus and suspended solids removal is accomplished by sand filtration of the secondary effluent. Total phosphorus is reduced by 80%, which results in effluent concentrations of less than 1 mg/L. Post aeration equipment is provided to increase the effluent DO concentration prior to discharge to Trail Creek. During the months of June through September (which coincides with the seeding of Trail Creek with smolts and later fish migration up Trail Creek), a pure

oxygen system supersaturates the plant effluent to a DO concentration in excess of 13.0 mg/L.

Toxicity Identification Evaluation

Initial Toxicity Characterization

When both acute and chronic toxicity requirements must be met, POTW staff must decide whether to use acute or chronic TIE procedures to determine the effluent toxicants. Acute TIE procedures can be used to provide information about the causes of chronic toxicity and may be preferred because they are simpler and less costly than chronic TIE tests. Follow-up confirmation tests can be performed using chronic TIE procedures to determine if additional toxicants are contributing to chronic toxicity. If an effluent exhibits marginal and intermittent acute toxicity, it may not be possible to identify the causes of effluent toxicity using acute TIE procedures. In this case, chronic TIE procedures should be used.

The initial TIE work at the MCWTP focused on characterizing the causes of acute effluent toxicity because previous testing indicated that the effluent exhibited consistent acute toxicity. *C. dubia* were used as the test organism based on previous tests showing it to be more sensitive to the MCWTP effluent than *P. promelas*.

The toxicity characterization tests conducted during the first quarter of the TIE program included the following effluent manipulations:

- Pressure filtration (1.0 µm filter).
- Submicron filtration (0.22 µm filter) following pressure filtration (performed on one sample)
- Aeration.
- C18 SPE following filtration.
- Cation resin treatment following filtration/C18 SPE treatment.
- Anion resin treatment following filtration/C18 SPE treatment.

As shown in Table E-1, the four effluent samples characterized from April through June 1991 were consistently toxic and the magnitude of toxicity was similar in each sample (1.5 to 2.5 TUa). Slight reductions in toxicity occurred following filtration and aeration and acute toxicity was completely removed by the C18 SPE column. Toxicity removal by the cation and anion resins could not be determined because the sample was first passed through the C18 SPE column, which removed all of the toxicity. In retrospect, it would have been preferable to treat the samples with the ion exchange resins following filtration rather than after C18 SPE treatment. Relatively nonpolar organic compounds are preferentially adsorbed onto the C18 SPE column; therefore, toxicity removal by the C18

Table E-1. Acute Toxicity Characterization Test Results from April 1991 Through June 1991

Characterization Test	*C. dubia* LC50 (TUa)*			
	4/18/91	5/16/91	6/5/91	6/19/91
Baseline (whole effluent)	42 (2.4)	40 (2.5)	46 (2.2)	67 (1.5)
Filtration	51 (2.0) †	79 (1.3) ‡	54 (1.9) †	§
Aeration ω	40 (2.5)	62 (1.6)	51 (2.0)	§
Post C18 SPE ι	>100 (0.0)	>100 (0.0)	>100 (0.0)	>100 (0.0) #
Cation exchange τ	>100 (0.0)	>100 (0.4)	>100 (0.0)	§
Anion exchange ¢	>100 (0.0)	>100 (0.0)	>100 (0.2)	§

* *C. dubia* 48-hour LC50 values expressed as percent effluent with acute TUs (100/LC50) in parentheses.
† Effluent first pressure filtered through a Gelman A/E glass fiber filter (1.0 µm).
‡ Effluent first pressure filtered through a Gelman A/E glass fiber filter (1.0 µm), followed by filtration through a Micro Separation, Inc., 0.22 µm nylon filter.
§ Characterization manipulation not conducted.
# Fine stream of air bubbles passed through an effluent sample placed in a graduated cylinder.
ω Effluent sequentially pressure filtered (1.0 µm) and passed over a C18 SPE column.
ι Effluent passed directly over a C18 SPE column.
τ Effluent passed over a Bio-Rex MSZ 50 cation resin after pressure filtration and C18 SPE treatment.
¢ Effluent passed over a Bio-Rex MSZ 1 anion resin after pressure filtration and C18 SPE treatment.

SPE treatment during the initial characterization tests suggested that non-polar or semi-polar organic compounds were causes of effluent toxicity.

Evaluation of Toxicity Removed by C18 SPE

The C18 SPE column can remove toxicants other than non-polar organic compounds, including organometallic complexes, certain metal ions, surfactants, and some high molecular weight organic compounds. Accordingly, additional tests were performed from July through October 1991 to obtain information about the types of compounds removed by the C18 SPE treatment. In an attempt to recover toxicity from the C18 SPE column, sequential elutions were performed with methanol, methylene chloride, 3N hydrochloric acid, and 9N sodium hydroxide. Metals were evaluated as possible causes of toxicity concurrently with the C18 SPE tests. Metals toxicity was investigated by adding EDTA to whole effluent samples and testing for acute toxicity. EDTA forms complexes with many toxic metals and, when added at appropriate concentrations, can render metals non-toxic.

Results of the C18 SPE column and EDTA tests are summarized in Table E-2. In contrast to previous tests, the acute toxicity of the whole effluent from August through October 1991 was variable and intermittent (Table E-2). Four of the seven effluent samples were not acutely toxic. The three acutely toxic samples were rendered non-toxic by the C18 SPE treatment; however, toxicity was not recovered by eluting the C18 SPE columns with methanol, methylene chloride, 3N hydrochloric acid, or 9N sodium hydroxide. Toxicity could not be successfully eluted from C18 SPE columns using conventional organic extraction techniques; therefore, it was concluded that the toxicity removed by the column was not caused by typical non-polar or semi-polar organic compounds.

Addition of EDTA to the three acutely toxic samples eliminated acute toxicity, suggesting that toxicity was caused by metals. The EDTA results provide evidence that the toxicity removed by the C18 SPE column was not caused by non-polar or semi-polar organic compounds. Instead, it indicated that metals or organometallic complexes were removed in the C18 SPE column tests. These results demonstrate the importance of needing to recover toxicity from the C18 SPE column before concluding that non-polar organic compounds are a cause of effluent toxicity.

Evaluation of Metal Toxicity

Additional testing was performed to evaluate metals as a cause of chronic effluent toxicity to *C. dubia*. Chronic tests were used to help avoid problems associated with the intermittent acute toxicity; however, acute toxicity endpoints (e.g., 48-hour LC50) were also obtained from the chronic tests. During October 1991 through January 1992, 7-day static renewal *C. dubia* survival and reproduction tests were performed on whole effluent samples and whole

Table E-2. Toxicity Characterization Test Results from July 1991 Through October 9, 1991

Sample Date	*C. dubia* LC50 (TUa) *		
	Baseline (Final Effluent)	Post C18 SPE †	EDTA ‡
7/10/91	>100 (0.2)	§	§
7/24/91	>100 (0.0)	>100 (0.2)	>100 (0.0)
8/07/91	61 (1.6)	>100 (0.0)	>100 (0.0)
8/22/91	52 (1.9)	>100 (0.4)	>100 (0.0)
9/11/91	>100 (0.4)	§	§
9/25/91	>100 (0.2)	>100 (0.0)	>100 (0.0)
10/09/91	<100 (>1) #	§	>100 (0.0) ω

* *C. dubia* 48-hour LC50 values expressed as percent with TUs (100/LC50) in parentheses.
† Effluent passed over a C18 SPE column.
‡ EDTA was added to the final effluent at a concentration of 186 mg/L.
§ Characterization manipulation was not conducted.
# Test conducted only in 100% effluent; as a result, LC50 and TUa values could not be calculated.
ω EDTA concentration in the 10/09/91 sample was 18.6 mg/L.

effluent samples with EDTA added. As shown in Table E-3, three of the five samples exhibited acute toxicity and four of the five were chronically toxic. The 48-hour LC50 values for all of the EDTA treated samples were greater than 100% effluent. EDTA addition also eliminated chronic toxicity in two samples and reduced chronic toxicity in a third sample. These results provided additional evidence that metals cause acute effluent toxicity, and also suggested that metals were a primary cause of chronic effluent toxicity.

The correlation approach and spiking approach described by USEPA (1989a) were used to confirm that metals were causing effluent toxicity. The correlation approach is intended to evaluate the relationship between the concentration of suspected toxicants and effluent toxicity. Toxicity and metals data (aluminum, Cd, Cu, Ni, and Zn) for six effluent samples were compared by correlation analysis. All metals were measured as total metals.

Linear regression analysis indicated a good correlation (regression coefficient of 0.72) between effluent toxicity and effluent Cd concentrations. However, when data from May 1991 through December 1992 were pooled with the data set, the correlation between effluent toxicity and effluent Cd concentrations was not statistically significant. A comparison of the mean Cd concentrations from samples collected during a toxic period (May 1991 to December 1991), and those taken during a non-toxic period (May 1992 to December 1992) indicated a trend. The mean Cd concentration was 4.1 µg/L during the toxic period and 0.47 µg/L during the non-toxic period. These data provide evidence that Cd was contributing to effluent toxicity. No significant correlation was observed between effluent toxicity and the concentration of the other metals or the sum of all the metals.

The objective of the spiking approach was to determine whether an increase in the concentration of a suspected toxicant would cause a proportional increase in toxicity. Chronic *C. dubia* toxicity tests were performed on three chronically toxic effluent samples both with and without added Cd, Cu, Ni, and Zn. The metals were added in nominal concentrations approximating those typically found in the MCWTP effluent. The results indicated that effluent toxicity did not consistently increase when the metals were spiked individually or in combination. Therefore, the results of the spiking tests did not confirm that Cd or other metals were contributing to effluent toxicity.

Toxicity Control Evaluation and Implementation

Although the TIE did not conclusively identify the specific causes of effluent toxicity, the weight of evidence indicated that effluent toxicity was caused by metals. As a result, Michigan City investigated possible sources of metals in the collection system. Pretreatment program data indicated that a cadmium plating facility in the MCWTP service area was consistently out of compliance with pretreatment limitations for metals. Based on the persistent pretreatment permit violations, the cadmium plating

Table E-3. Acute and Chronic Toxicity of MCWTP's Effluent (with and without added EDTA) from October 1991 Through January 1992

	Final Effluent		Final Effluent with EDTA Added *	
Sample Date	Acute LC50 (TUa) †	Chronic NOEC ‡	Acute LC50 (TUa) †	Chronic NOEC ‡
10/30/91	73 (1.4)	50	>100 (0.0)	100
11/14/91	>100 (0.0)	62	>100 (0.0)	62
12/04/91	>100 (0.0)	100	>100 (0.0)	100
12/18/91	84 (1.2)	<50	>100 (0.0)	100
01/08/92	60 (1.7)	<50	>100 (0.0)	50

* EDTA concentration in the 10/30/91 and 11/14/91 tests was 5 mg/L. EDTA concentration in the 12/04/91, 12/18/91, and 01/08/92 tests was 10 mg/L.

† *C. dubia* 48-hour LC50 values expressed as percent effluent with acute TUas (100/LC50) in parentheses.

‡ Reproduction NOEC values expressed as percent effluent calculated from 7-day static-renewal chronic tests with *C. dubia*.

company was issued a consent decree to terminate their cadmium plating operation. The cadmium plating operation was shut down in April 1992.

The impact of the shutdown on effluent toxicity was evaluated by performing 4-day modified chronic *C. dubia* tests on whole effluent samples at approximately 2-week intervals from May through September 1992 (total of nine tests). The 4-day modified chronic tests consisted of four concentrations and a control, five replicate test chambers per concentration, and the tests were initiated with 3-day old *C. dubia*. This modified approach has been demonstrated to produce results that are comparable to the 7-day test (Masters et al., 1991). The results of these tests showed that acute and chronic effluent toxicity to *C. dubia* had been eliminated.

Discussion

Subsequent chronic testing with *C. dubia* and *P. promelas* using compliance monitoring procedures (USEPA, 1989b) confirmed the reduction in effluent toxicity following shutdown of the cadmium plating operation. The acute and chronic toxicity of the MCWTP effluent from inception of the TRE through December 1992 is summarized in Figure E-1. The correlation between the cadmium plating operation shutdown and improved effluent toxicity is clearly evident. Based on the improved effluent toxicity, the TRE was terminated and semiannual acute and chronic toxicity compliance monitoring was initiated.

However, starting in August 1996 significant reproductive effects were observed in 100% effluent as compared to the test control. Subsequent TIE testing was inconclusive because effluent samples were nontoxic. Michigan City has submitted a letter to the Indiana Department of Environmental Management (IDEM) requesting changes in the effluent monitoring program. The requested changes include the use of reconstituted laboratory water as dilution water in lieu of receiving water to minimize potential contamination and reducing the frequency of monitoring if no toxicity is observed in three consecutive tests. As of October 1997, a decision from IDEM was still pending.

Acknowledgments

This TIE effort was conducted by Great Lakes Environmental Center, Traverse City, Michigan, under contract to the Michigan City Sanitary District.

References

Masters, J.A., M.A. Lewis, D.H. Davidson, and R.D.

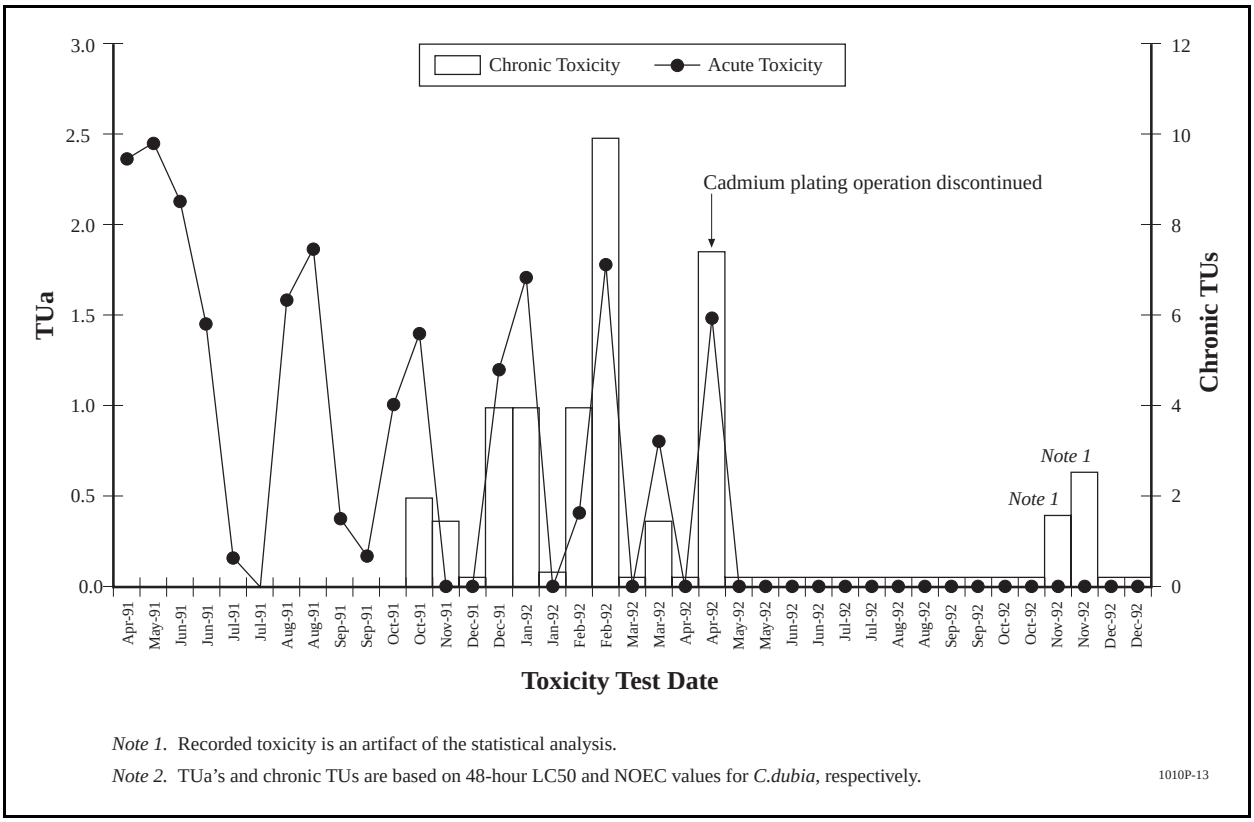

Figure E-1. Acute and chronic effluent toxicity: 1991 through 1992.

Bruce. 1991. "Validation of a Four-Day *C. dubia* Toxicity Test and Statistical Considerations in Data Analysis" *Environ. Toxicol. Chem*. 10: 47-55.

Michigan City Sanitary District (MCSD). 1993. *Draft Final Report on Toxicity Identification/Reduction Evaluation for Michigan City Wastewater Treatment Plant*. Prepared by Great Lakes Environmental Center, Traverse City, Michigan.

USEPA. 1989a. *Methods for Aquatic Toxicity Identification Evaluations: Phase III Toxicity Confirmation Procedures*. EPA/600/3-88/036. Office of Research and Development, Duluth, Minnesota.

USEPA. 1989b. *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*. Second Edition. EPA/600/4-89/001. Office of Research and Development, Cincinnati, Ohio.